FEBS Letters 585 (2011) 1163–1168

journal homepage: www.FEBSLetters.org

The binding of 14-3-3γ to membranes studied by intrinsic fluorescence spectroscopy

Helene J. Bustad [a,b], Jarl Underhaug [a], Øyvind Halskau Jr. [a,c], Aurora Martinez [a,*]

[a] Department of Biomedicine, University of Bergen, Jonas Lies vei 91, N-5009 Bergen, Norway
[b] Norwegian Porphyria Centre (NAPOS), Laboratory of Clinical Biochemistry, Haukeland University Hospital, Bergen, Norway
[c] Department of Molecular Biology, University of Bergen, Thormøhlensgate 55, N-5020 Bergen, Norway

ARTICLE INFO

Article history:
Received 14 February 2011
Revised 11 March 2011
Accepted 14 March 2011
Available online 21 March 2011

Edited by Sandro Sonnino

Keywords:
Cell signalling
Tryptophan fluorescence
Membrane-binding
Acidic liposomes
Quenching
Pi-stacking

ABSTRACT

Human 14-3-3 proteins contain two conserved tryptophan residues in each monomer, Trp60 and Trp233 in isoform γ. 14-3-3γ binds to negatively charged membranes and here we show that membrane binding can be monitored by steady-state intrinsic fluorescence spectroscopy. Measurements with W60F and W233F 14-3-3γ mutants revealed that Trp60 is the major contributor to the emission fluorescence, whereas the fluorescence of Trp233, which π-stacks with Tyr184, is quenched. The fluorescence is reduced and red-shifted upon specific binding of a phosphate ligand, and further red-shifted upon binding of 14-3-3γ to the membrane, compatible with solvent exposure of Trp60. Moreover, our results support that membrane binding involves the non-conserved, convex area of 14-3-3γ, and that Trp residues do not intercalate in the bilayer.

1. Introduction

Integral or peripheral membrane proteins may be experimentally difficult to investigate due to the challenges imposed by the membranes or bilayer-mimicking systems in the analysis, greatly restricting the choice of methods to study protein–membrane interactions. Fluorescence spectroscopy is a versatile method with high sensitivity, used to study and track differences between protein states, and it has also been applied to characterize protein–membrane binding [1,2]. Fluorescence requires low concentrations of protein and thus relatively low absolute lipid concentrations to reach saturating protein binding conditions. Tryptophan (Trp), with an emission maximum (λ_{max}) around 355 nm when fully exposed [2], is a naturally occurring fluorophore in proteins and is commonly used in studies of protein folding, binding, and dynamics, as the Trp fluorescence intensity and λ_{max} are very sensitive to the polarity of the solvent and the local protein environment. A red shift (to longer wavelengths) in λ_{max} is associated with the transition of Trp residues from a hydrophobic to a more polar environment. Red shifts are often related to the protein undergoing conformational changes, but can also be related to intrinsic changes that promote protein binding to membranes and to the protein state induced by binding to bilayers. Such changes have been well characterized for the interaction of α-lactalbumin (α-LA) with membranes [3,4]. Trp has also been used to track the protein state and membrane-binding status for other amphitropic proteins, such as cytochrome c [5], α-sarcin [6], and α-synuclein [7].

The dimeric 14-3-3 proteins are ubiquitously expressed in all eukaryotic cells, and found as seven isoforms in humans (as reviewed in [8]). The ubiquity and high degree of conservation is compatible with their fundamental importance in the eukaryotic cell, and the proteins are – mainly through interaction with phosphorylated protein partners – involved in numerous cellular processes [8,9]. The 14-3-3 proteins are regarded as soluble, promoting nucleo-cytoplasmic localization of target proteins [10–13], but association of some isoforms with membranes in chromaffin cells and synaptic vesicles has been reported [14–17]. Recently, we have proposed that the peripheral binding of the γ isoform to negatively charged membranes could be associated to a role in shuttling interaction partners, such as phosphorylated tyrosine hydroxylase (TH), to the membrane [18].

We here present a study on the binding of 14-3-3γ to membranes based on the binding-induced changes in the intrinsic fluorescence of the protein. We have prepared Trp → Phe mutants of the two Trp residues in 14-3-3γ, i.e., Trp60 and Trp233, in order to elucidate the contribution of the individual Trp residues to the total intrinsic fluorescence of 14-3-3γ and to obtain insights into the structural determinants for membrane-binding. The results

* Corresponding author. Fax: +47 55586360.
E-mail address: aurora.martinez@biomed.uib.no (A. Martinez).

0014-5793/$36.00
doi:10.1016/j.febslet.2011.03.027

obtained identify Trp60 as the main contributor to the intrinsic fluorescence emission of 14-3-3γ, and provide insights into the structural basis of the protein membrane association.

2. Materials and methods

2.1. Phospholipids and liposomes

Phosphatidylcholine (PC) from egg yolk lecithin (95% PC) was purchased from Sigma–Aldrich and porcine brain phosphatidylserine (PBPS) was from Avanti Polar Lipids, Inc. Liposomes were prepared as large unilamellar vesicles, made of either PC alone or of PC:PBPS mixtures (1:1) as described [18].

2.2. Site-directed mutagenesis, expression and purification

The missense mutations in Trp60 and Trp233 were introduced into the 14-3-3γ cDNA on the pGEX-expression vector using the QuikChange® site-directed mutagenesis kit (Stratagene®). To verify the mutations, the entire gene was sequenced. Wild-type (wt) and mutants were expressed in *Escherichia coli* BL-21-CodonPlus® as glutathione S-transferase fusion proteins, and further purified as reported [18]. To ensure complete depletion of protein-bound phosphate prior to some experiments, as indicated, extensive dialysis of the 14-3-3γ proteins against 10 mM HEPES, pH 6.0, was performed at 4 °C.

2.3. Intrinsic fluorescence spectroscopy

Fluorescence measurements were performed at 25 °C using a LS50B Perkin Elmer luminescence spectrometer with 14-3-3γ initially prepared in 10 mM HEPES, pH 6.0, or 100 mM Na-phosphate, pH 6.0. This pH was selected because preliminary data showed higher binding at pH 6.0 than 7.0 for 14-3-3γ, in accordance with pH-dependent binding experiments by surface plasmon resonance (SPR) [18]. Final protein concentration was 1.6–2.0 μM, and all data was normalized with respect to concentration. An excitation wavelength of 295 nm was used, and excitation and emission slits were set to 3 nm and 5 nm, respectively. Emission spectra were collected at 100 nm/min in the range 320–400, and 2–3 sample parallels were acquired. Spectra of blank samples were subtracted from the main spectra.

To avoid error and bias in assigning λ_{max} values, the fluorescence spectra were fitted to a four-parameter log-normal function of the following form [19]:

$$I(v) = \begin{cases} I_m \exp\left(-\frac{\ln 2}{\ln^2 \rho} \ln^2 \frac{a-v}{a-v_m}\right) & \text{when } v < a, \\ 0 & \text{when } v \geq a, \end{cases} \quad (1)$$

where I_m is the maximum fluorescence intensity, v_m is the wave number of the maximum, ρ is an asymmetry parameter, and α is the function limiting point.

3. Results and discussion

3.1. The fluorescence emission spectra of 14-3-3γ

The fluorescence emission spectrum of 14-3-3γ in the absence of liposomes in Na-phosphate showed a λ_{max} of 342.6 nm and fluorescence intensity just below 17 a.u./μM (see Fig. 1A). In the presence of negatively charged PC:PBPS liposomes, the spectrum revealed a red shift towards longer λ_{max} (Fig. 1A, B). Moreover, a decrease in intensity was also seen upon liposome addition (Fig. 1A). No noticeable red shift was observed in the presence of uncharged PC liposomes, and the intensity decrease is also smaller in the presence of PC liposomes than with PC:PBPS liposomes (Fig. 1A). As seen earlier by SPR, 14-3-3γ shows no noticeable binding to liposomes made of neutral phospholipids such as PC [18]. Thus, a red shift of the emission spectrum appears as an appropriate parameter to monitor the binding of 14-3-3γ to negatively charged membranes. The accompanying quenching in fluorescence intensity is partly due to high scattering and lowered light transmission in the presence of the liposomes. However, the larger quenching observed in the presence of PC:PBPS liposomes compared to neutral liposomes at equal phospholipid/protein concentration (Fig. 1A) appears to be accompanied by a solvent exposure of the fluorophores in the protein, with enhanced access to quenchers like O_2 [20].

Previous results indicated that 14-3-3γ increases its affinity for negatively charged membranes when it has cargo, such as the phosphorylated peptide corresponding to the N-terminal region of TH (THp1-43) [18]. We have later observed by SPR that 14-3-3γ thoroughly dialyzed in phosphate-free buffer (d14-3-3γ) does not bind to negatively charged membranes, while the binding is recovered when phosphorylated peptides or inorganic phosphate is added to the dialyzed protein (non-published results). We therefore proceeded to investigate the effect of phosphate on the emission fluorescence spectrum and the titration with liposomes. As seen in Fig. 1A the spectrum of d14-3-3γ is characterized by a similar λ_{max} (342.1 nm) and a ~9 a.u./μM higher fluorescence intensity than the protein in Na-phosphate (Fig. 1A). Interestingly, upon titration of the dialyzed sample with PC:PBPS liposomes, no red shift of λ_{max} indicative of binding was obtained (Fig. 1B), and d14-3-3γ behaved in this respect rather similarly to 14-3-3γ with neutral PC liposomes. Altogether the results show that the phosphate-dependent binding of 14-3-3γ to negatively charged membranes can be monitored by fluorescence spectroscopy. Preliminary titrations with 14-3-3ζ indicated no binding, as no red shift was observed (data not shown), compatible with isoform-specific membrane binding, as previously seen by SPR, where 14-3-3γ revealed much higher membrane affinity compared to 14-3-3ζ [18]. As Trp60 and Trp233 are conserved in all seven 14-3-3 human isoforms, fluorescence spectroscopy is expected to be applicable to probe membrane binding for all subtypes.

3.2. Tryptophan → phenylalanine mutants

In order to examine the individual contribution of each Trp residue (Trp60 and Trp233) to the emission fluorescence of 14-3-3γ and to identify the residue(s) responsible for the spectroscopic changes induced by membrane binding, we generated mutants in which each one of the two Trp residues were changed into Phe and studied their spectroscopic properties. The emission spectrum of W233F (Fig. 2A) containing only Trp60 in Na-phosphate, resembled that of wt, only revealing a slightly lower fluorescence intensity but similar λ_{max} in absence of liposomes (342.1 nm). Similar to what was observed for the wt protein (Fig. 1A) dialysis of the W233F mutant against phosphate-free buffer (providing dW233F) resulted in increased fluorescence intensity (Fig. 2A), consistent with a decreased solvent exposure of Trp60 in the dialyzed protein. But in the case of the mutant a ~4-nm blue-shift was measured after dialysis (Fig. 2A), further corroborating that phosphate binding induces conformational changes that increase the solvent exposure of Trp60. The emission spectrum of dW233F only experienced a decrease in intensity upon addition of liposomes made of PC:PBPS (Fig. 2A), and the λ_{max} was relatively stable upon titration (Fig. 2B), following the same pattern as wt (Fig. 1B). The spectrum of W233F in phosphate, however, experienced both the decrease in fluorescence intensity in the presence of negatively charged liposomes (Fig 2A), and a red shift dependent on liposome content (Fig. 2B).

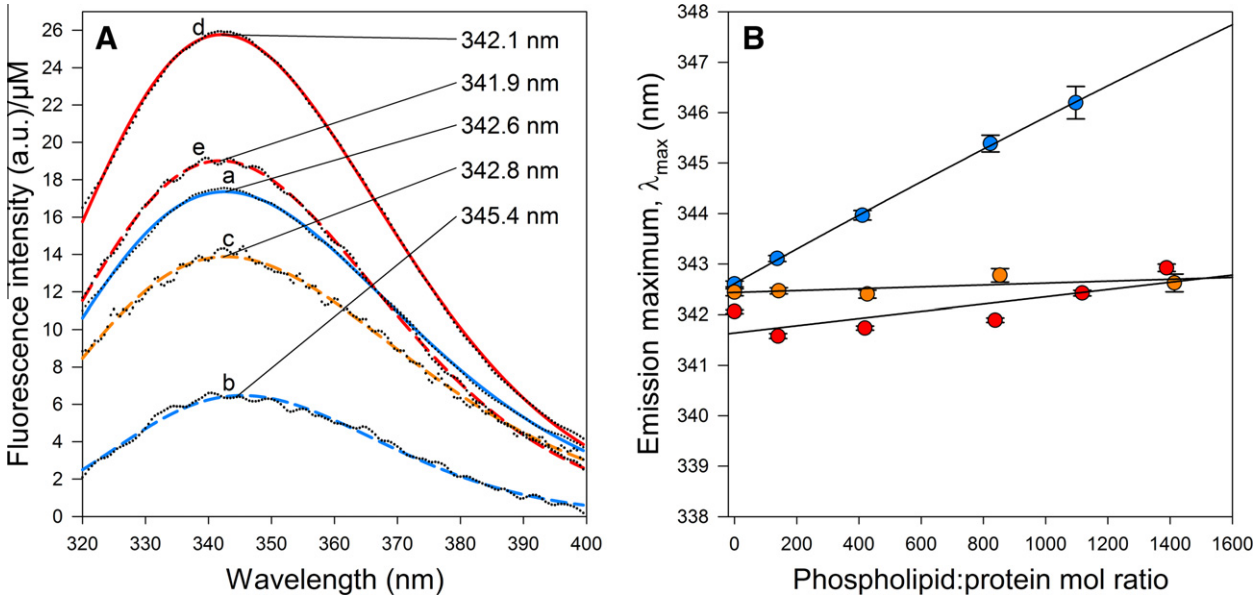

Fig. 1. The membrane binding ability of 14-3-3γ and the effect of phosphate. (A) The emission spectra of 14-3-3γ, in 100 mM Na-phosphate, pH 6.0 in the absence (a), and presence of liposomes of PC:PBPS (b) or PC (c) at phospholipid:protein mol ratio of ~830:1; the emission spectra of 14-3-3γ dialyzed and prepared in 10 mM HEPES, pH 6.0 (d14-3-3γ) in the absence (d) and presence of liposomes made of PC:PBPS at phospholipid:protein mol ratio of ~800:1 (e). The black dots represent the data points and the colored lines are for the fittings. (B) Effect on the λ_{max} of 14-3-3γ with increasing PC:PBPS (blue) or PC (ochre) liposome concentration, and of d14-3-3γ with PC:PBPS liposomes (red).

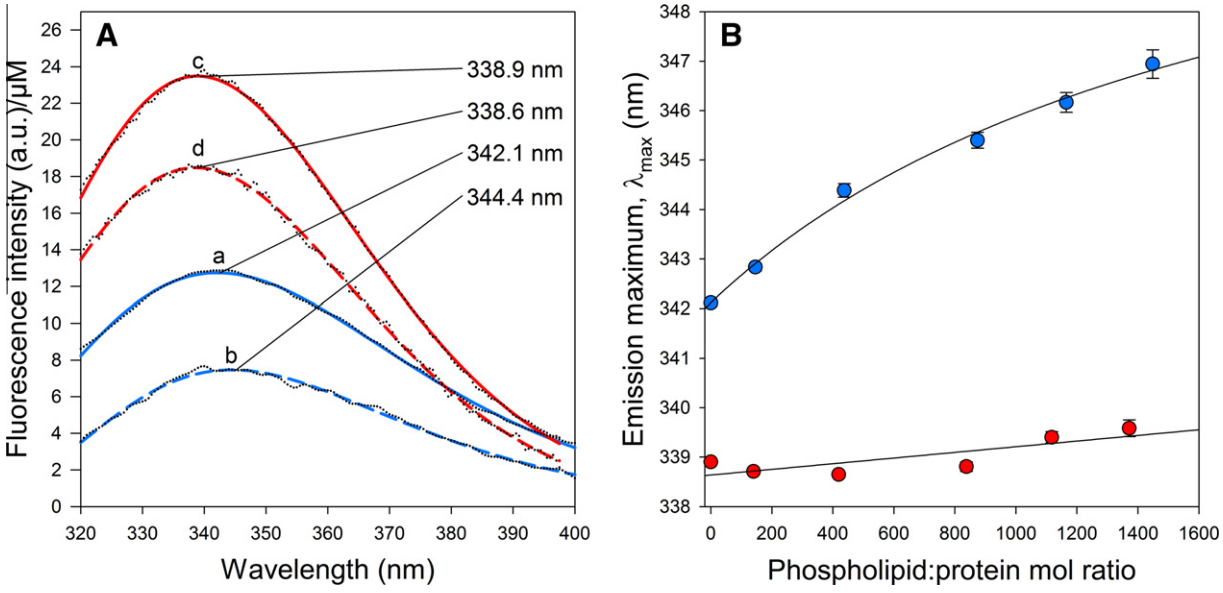

Fig. 2. Effect of the mutation W233F on the emission fluorescence spectrum; titration with liposomes. (A) The emission spectra of W233F, in 100 mM Na-phosphate, pH 6.0 in the absence (a) and presence of PC:PBPS liposomes (b) at phospholipid:protein mol ratio of ~430:1; the emission spectra of dW233F, dialyzed and prepared in 10 mM HEPES, pH 6.0 in the absence (c) and presence of PC:PBPS liposomes at phospholipid:protein mol ratio of ~420:1 (d). Data points presented with black dots and fittings with colored lines. (B) Effect on the λ_{max} of W233F (blue) or dW233F (red) with increasing PC:PBPS liposome concentration.

Based on the fluorescence properties of W233F relative to wt, we expected that the mutant W60F, containing only Trp233, would reveal low emission fluorescence. But in fact the fluorescence emission of W60F was nearly absent throughout the wavelength measuring interval, also when liposomes were present (Fig. 3A). Histidine and tyrosine may quench the fluorescence of tryptophan, as e.g. seen for the pH dependent fluorescence of Trp94 in barnase, which is effectively quenched by protonated His18 [21]. As revealed by the crystal structure of 14-3-3γ complexed with the RAIpSLP phosphopeptide (PDB 2B05), no His residue is sufficiently close to Trp233 but we noted with interest that this residue interacted with Tyr184 through a π-stacking (Fig. 4A, C). This stacking of the rings might favor the optimal coupling for tyrosinate formation and quenching of the Trp fluorescence. Alternatively, the stacking might favor the orientation of the Trp rotamer for quenching of its fluorescence through electrostatically enabled electron transfer to other groups [22]. Supporting this possibility we found that increasing the pH of the sample had a remarkable effect on the emission fluorescence of the mutant W60F (Fig. 3A). The alkaline-dependent increase in fluorescence – associated with Trp233 – is also observed for the wt protein (Fig. 3B). The emission at alkaline pH is very red-shifted for both the mutant W60F (λ_{max} = 360 nm) and the wt (λ_{max} = 356 nm) (Fig. 3). The folding state of wt up to pH 10.0 was controlled by circular dichroism, showing that the

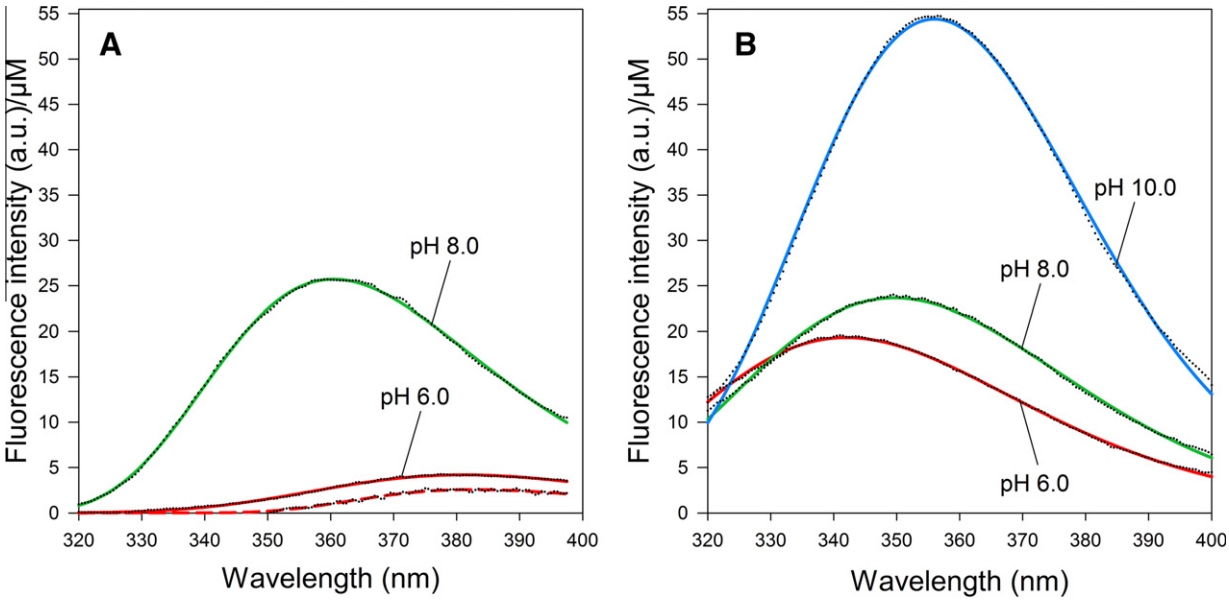

Fig. 3. Effect of the mutation W60F on the emission fluorescence spectrum; pH-effect on fluorescence intensity and λ_{max} values. (A) The W60F-mutant in 100 mM Na-phosphate, pH 6.0 both without (fitting in red continuous line) and with PC:PBPS liposomes at phospholipid:protein mol ratio of ~420:1 (dashed red line), and after increasing the pH from 6.0 to 8.0; λ_{max} = 360.5 nm (green line). (B) The pH-effect is also seen in the spectrum of wt, where both λ_{max} and fluorescence intensity increase with increasing pH from pH 6.0 (red; λ_{max} = 342.0 nm), to pH 8.0 (green; λ_{max} = 349.7 nm) and to pH 10.0 (blue; λ_{max} = 356.0 nm).

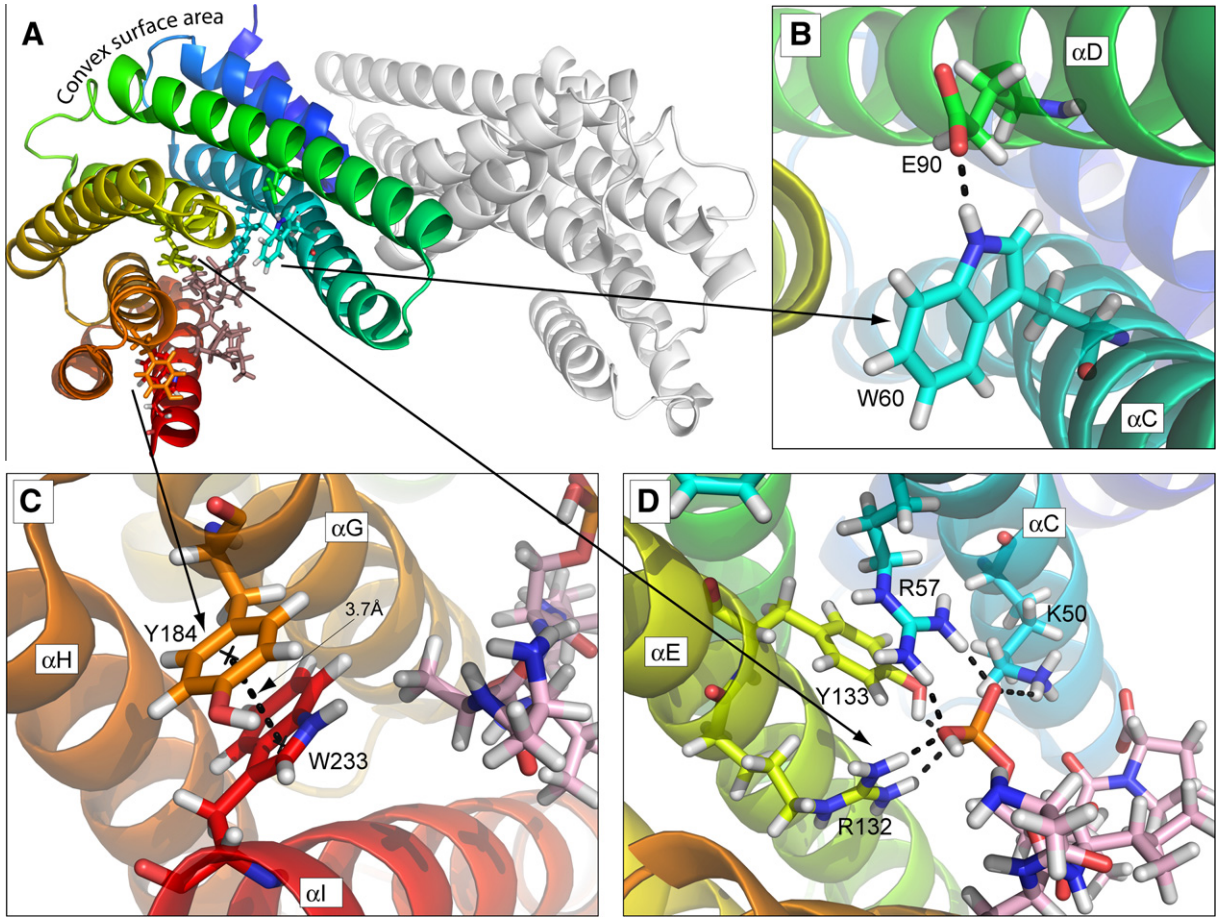

Fig. 4. Structural representation of ligand bound human 14-3-3γ (PDB 2B05). (A) Dimeric 14-3-3γ with one monomer in light gray and the other with the nine helices in rainbow colors from violet (αA) to red (αI), and the phosphopeptide ligand (RAIpSLP) shown in pink. Residues of interest are shown as sticks, and are highlighted in panels B, C and D. (B) The stabilizing hydrogen-bond (dashed line) between Trp60 (in αC) and Glu90 (αD) is disrupted when is mutated to Phe (W60F). (C) The π-stacking interaction between Trp233 (αI) and Tyr184 (αG) may silence the intrinsic fluorescence of Trp233 at λ_{ex} = 295 nm. (D) The phosphate of the ligand creates 6 hydrogen-bonds with several amino acids on αE (Arg132 and Tyr133) and αC (Lys50 and Arg57), creating a very stabilizing interaction network. The figures were prepared with PyMol [26].

increase in red-shifted fluorescence is not due to denaturation of the protein at high pH (Fig. S1 in Supplementary data). The high λ_{max} for the fluorescence emission of Trp233 is most probably associated to the effect of the stacking of Tyr184 in the tyrosinate form. Dialysis of the W60F mutant against phosphate-free buffer was attempted, but the dialyzed protein proved to be very unstable and precipitated, and was thus not recovered from the dialysis cassette (see below).

3.3. Structural and mechanistic implications for membrane binding

The evolution of the fluorescence intensity and λ_{max} for emission fluorescence in the mutant 14-3-3γ proteins indicates that Trp60 is very solvent exposed in ligand-free dW233F, and becomes even more solvent exposed upon phosphate binding. Additionally, the binding of the phosphate-bound 14-3-3γ (both wt and W233F) to negatively charged membranes results in a further solvent exposure of Trp60, in accordance with the membrane binding occurring through the convex surface of 14-3-3γ [16,18]. Proximity to the membrane may facilitate conformational changes on the convex surface causing the protein to further expose the bound phosphorylated cargo and Trp60, both at the concave area. Upon membrane binding, the red shift in λ_{max} is accompanied by the expected reduction in fluorescence intensity (Figs. 1A and 2A). Partial unfolding of the protein that occurs in other amphitropic interactions, with concomitant increase of emission intensity upon membrane-binding [4,23], does not appear to occur in the case of 14-3-3γ.

The structure of ligand-bound 14-3-3γ shows a very solvent exposed Trp60 (Fig. 4). The phosphate group in the phosphopeptide binds through six hydrogen-bonds with Tyr50, Lys57, Arg132 and Tyr133, conserved across the 14-3-3 human family and located at the other side of the channel with respect to Trp60, which is not directly involved in the interaction with the phosphate group (Fig. 4A, D). Comparison of the structure of the β-isoform of 14-3-3 in its ligand free (PDB 2BQ0) and peptide-bound (PDB 2C23) states [24] reveals that ligand binding leads to a packing and closure of the helices around the phosphate, leaving Trp60 slightly more solvent accessible, but without perturbing its hydrogen-bonding with Glu89 (Glu90 in 14-3-3γ; Fig. 4B). The stabilizing effect of this interaction is revealed by the instability of W60F and the large tendency to precipitate of dW60F, where the phosphate is removed by dialysis. This mutant, however, shows a stability that is close to wt when prepared in Na-phosphate. The highly stabilizing hydrogen-bond network established by phosphate (Fig. 4D) can most probably override the loss of the Trp60–Glu90 interaction in the W60F mutant.

Finally, our results do not support that membrane binding involves the intercalation of any of the Trp residues in the membrane. Intercalation of Trp60 would result in blue-shifts of the emission fluorescence of W233F as observed for many other membrane-binder proteins [7,25]. In the case of Trp233, an emission spectrum in the W60F is only recorded at pH $\geqslant$ 8 (Fig. 3A), where the affinity for the membrane is reduced [18]. Nevertheless, it is likewise expected that intercalation of Trp233 into the membrane might have relieved the internal quenching, which is not the case since the fluorescence of this residue is completely quenched throughout the titration with liposomes (Fig. 3A and data not shown).

4. Conclusions

We show in this work that intrinsic fluorescence spectroscopy may be used to monitor the phosphate/phosphopeptide-dependent membrane interaction of 14-3-3γ. This method is very sensitive, requiring low amounts of materials (proteins and phospholipidic membranes prepared as liposomes). Combined with site-directed mutagenesis at Trp residues, fluorescence spectroscopy provides information on the protein orientation and conformational changes associated with ligand binding and interaction with the membrane, and can probably be extended to study the interaction of 14-3-3 proteins with other partners. At neutral pH Trp60 is the main contributor to the intrinsic fluorescence in 14-3-3γ, while the fluorescence of Trp233 appears to be quenched due to the stacking interaction of this residue with Tyr184. The fluorescence of Trp60 is clearly red-shifted and quenched upon membrane association, indicative of further solvent exposure of Trp60. This finding would be compatible with an opening of the protein for exposure of the phosphorylated cargo upon interaction with the membrane through the convex side.

Acknowledgements

We are very grateful to Ali J. Sepulveda and Randi M. Svebak for expert technical assistance. This work was supported by grants from the Norwegian Cancer Society and the Research Council of Norway.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2011.03.027.

References

[1] Matos, P.M., Franquelim, H.G., Castanho, M.A. and Santos, N.C. (2010) Quantitative assessment of peptide–lipid interactions. Ubiquitous fluorescence methodologies. Biochim. Biophys. Acta 1798, 1999–2012.
[2] Munishkina, L.A. and Fink, A.L. (2007) Fluorescence as a method to reveal structures and membrane-interactions of amyloidogenic proteins. Biochim. Biophys. Acta 1768, 1862–1885.
[3] Agasøster, A.V., Halskau, Ø., Fuglebakk, E., Frøystein, N.A., Muga, A., Holmsen, H. and Martínez, A. (2003) The interaction of peripheral proteins and membranes studied with a–lactalbumin and phospholipid bilayers of various compositions. J. Biol. Chem. 278, 21790–21797.
[4] Banuelos, S. and Muga, A. (1995) Binding of molten globule-like conformations to lipid bilayers. Structure of native and partially folded a–lactalbumin bound to model membranes. J. Biol. Chem. 270, 29910–29915.
[5] Pinheiro, T.J., Elove, G.A., Watts, A. and Roder, H. (1997) Structural and kinetic description of cytochrome c unfolding induced by the interaction with lipid vesicles. Biochemistry 36, 13122–13132.
[6] Gasset, M., Mancheno, J.M., Laynez, J., Lacadena, J., Fernandez-Ballester, G., Martinez del Pozo, A., Onaderra, M. and Gavilanes, J.G. (1995) Thermal unfolding of the cytotoxin alpha-sarcin: phospholipid binding induces destabilization of the protein structure. Biochim. Biophys. Acta 1252, 126–134.
[7] van Rooijen, B.D., van Leijenhorst-Groener, K.A., Claessens, M.M. and Subramaniam, V. (2009) Tryptophan fluorescence reveals structural features of alpha-synuclein oligomers. J. Mol. Biol. 394, 826–833.
[8] Aitken, A. (2006) 14-3-3 proteins: a historic overview. Semin. Cancer Biol. 16, 162–172.
[9] Fu, H., Subramanian, R.R. and Masters, S.C. (2000) 14-3-3 proteins: structure, function, and regulation. Annu. Rev. Pharmacol. Toxicol. 40, 617–647.
[10] Hekman, M. et al. (2006) Reversible membrane interaction of BAD requires two C-terminal lipid binding domains in conjunction with 14-3-3 protein binding. J. Biol. Chem. 281, 17321–17336.
[11] Lin, H.K. et al. (2009) Phosphorylation-dependent regulation of cytosolic localization and oncogenic function of Skp2 by Akt/PKB. Nat. Cell. Biol. 11, 420–432.
[12] Muslin, A.J. and Xing, H. (2000) 14-3-3 proteins: regulation of subcellular localization by molecular interference. Cell. Signal. 12, 703–709.
[13] Peterfy, M., Harris, T.E., Fujita, N. and Reue, K. (2010) Insulin-stimulated interaction with 14-3-3 promotes cytoplasmic localization of lipin-1 in adipocytes. J. Biol. Chem. 285, 3857–3864.
[14] Martin, H., Rostas, J., Patel, Y. and Aitken, A. (1994) Subcellular localisation of 14-3-3 isoforms in rat brain using specific antibodies. J. Neurochem. 63, 2259–2265.
[15] Roth, D., Morgan, A., Martin, H., Jones, D., Martens, G.J., Aitken, A. and Burgoyne, R.D. (1994) Characterization of 14-3-3 proteins in adrenal chromaffin cells and demonstration of isoform-specific phospholipid binding. Biochem. J. 301 (Pt 1), 305–310.

[16] Jones, D.H. et al. (1995) Expression and structural analysis of 14-3-3 proteins. J. Mol. Biol. 245, 375–384.
[17] Strochlic, L., Cartaud, A., Mejat, A., Grailhe, R., Schaeffer, L., Changeux, J.P. and Cartaud, J. (2004) 14-3-3 gamma associates with muscle specific kinase and regulates synaptic gene transcription at vertebrate neuromuscular synapse. Proc. Natl. Acad. Sci. USA 101, 18189–18194.
[18] Halskau Jr., Ø., Ying, M., Baumann, A., Kleppe, R., Rodriguez-Larrea, D., Almas, B., Haavik, J. and Martinez, A. (2009) Three-way interaction between 14-3-3 proteins, the N-terminal region of tyrosine hydroxylase, and negatively charged membranes. J. Biol. Chem. 284, 32758–32769.
[19] Burstein, E.A. and Emelyanenko, V.I. (1996) Log-normal description of fluorescence spectra of organic fluorophores. Photochem. Photobiol. 64, 316–320.
[20] Lakowicz, J.R. (2006) Principles of Fluorescence Spectroscopy, Springer, New York.
[21] Loewenthal, R., Sancho, J. and Fersht, A.R. (1991) Fluorescence spectrum of barnase: contributions of three tryptophan residues and a histidine-related pH dependence. Biochemistry 30, 6775–6779.
[22] Callis, P.R., Petrenko, A., Muino, P.L. and Tusell, J.R. (2007) Ab initio prediction of tryptophan fluorescence quenching by protein electric field enabled electron transfer. J. Phys. Chem. B 111, 10335–10339.
[23] Halskau, O., Frøystein, N.A., Muga, A. and Martínez, A. (2002) The membrane-bound conformation of alpha-lactalbumin studied by NMR-monitored 1H exchange. J. Mol. Biol. 321, 99–110.
[24] Yang, X. et al. (2006) Structural basis for protein–protein interactions in the 14-3-3 protein family. Proc. Natl. Acad. Sci. USA 103, 17237–17242.
[25] Zhao, H. and Kinnunen, P.K. (2002) Binding of the antimicrobial peptide temporin L to liposomes assessed by Trp fluorescence. J. Biol. Chem. 277, 25170–25177.
[26] Schrodinger, L.L.C. (2010) The PyMOL Molecular Graphics System, Version 1.3r1.